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PROJECT PRE-DISSERTATION REPORT BY DIMPY
FOR THE AWARD OF DIGREE OF M.Sc. BIOTECHNOLOGY
DEPARTMENT OF BIOTECHNOLOGY
LOVELY PROFESSIONAL UNIVERSITY

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TOPIC

**“IDENTIFICATION AND CHARACTERIZATION OF MICROBIAL
CONTAMINANTS AND THEIR EFFECTS IN PRODUCTION OF MILK
PRODUCTS”**

Declaration

I Dimpy hereby declare that the project report entitled “Identification and Characteristiczation of Microbial Contaminants and their Effect on Milk Products” submitted for the partial fulfilment of the degree M.Sc. Biotechnology is record of work carried out by me under the supervision of “Dr Mohammad Murtaza Mehdi”, Assistant Professor, Lovely Professional University, Phagwara, Punjab.

I further declare that the material taken from other sources have been duly acknowledged in this report.

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CERTIFICATE

This is to certify that Dimpy (Regis. No.) 11613865 have completed the project, entitled **“Identification and characterization of microbial contaminants and their effect on milk products”** under my guidance and supervision. To the best of my knowledge, the present work is the result of their original investigation and study.

No part of the report has ever been submitted for any other degree at any University. The report is fit for submission and the partial fulfilment of the conditions for the award of Master of Science in Biotechnology (Hons.)

Date:

Supervisor Signature:

Acknowledgement

I would never have been able to complete my writing without the guidance of my teacher, my friends and support from my family. I am heartily thankful to my supervisor Dr. Mohammad Murtaza Mehdi, Department of Biochemistry for encouragement and support me to understand this project. It is with my deepest gratitude and warmest affection that I dedicated this work to my sir, who has been a constant source of knowledge and inspiration and whose innovation and valuable ideas helped me to complete my work.

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INTRODUCTION

Crude milk can frequently be degraded with pathogens, either straightforwardly through living beings shed because of udder disease or by implication.

Roundabout defilement may emerge from

- (I) Dairy animals' own particular fecal issue sulling the udder and nipples
- (II) Fecal matter of different bovines debasing the udder
- (III) Milking groups reaching surfaces with fecal pollution, and
- (IV) Post-reap ecological tainting

A concentrated search of distributed and unpublished writing demonstrates that globally, crude dairy animals' milk is regularly tainted with pathogens and, while information is rare, the information which is accessible affirms that crude cow milk is a wellspring of pathogenic microorganisms at low levels.

Crude milk is exceedingly perishable, with both pathogenic and waste creatures anticipated that would multiply pending the receipt of test outcomes. Low levels of pathogens in crude milk display a hazard to purchasers, and much of the time these are beneath the levels of identification.

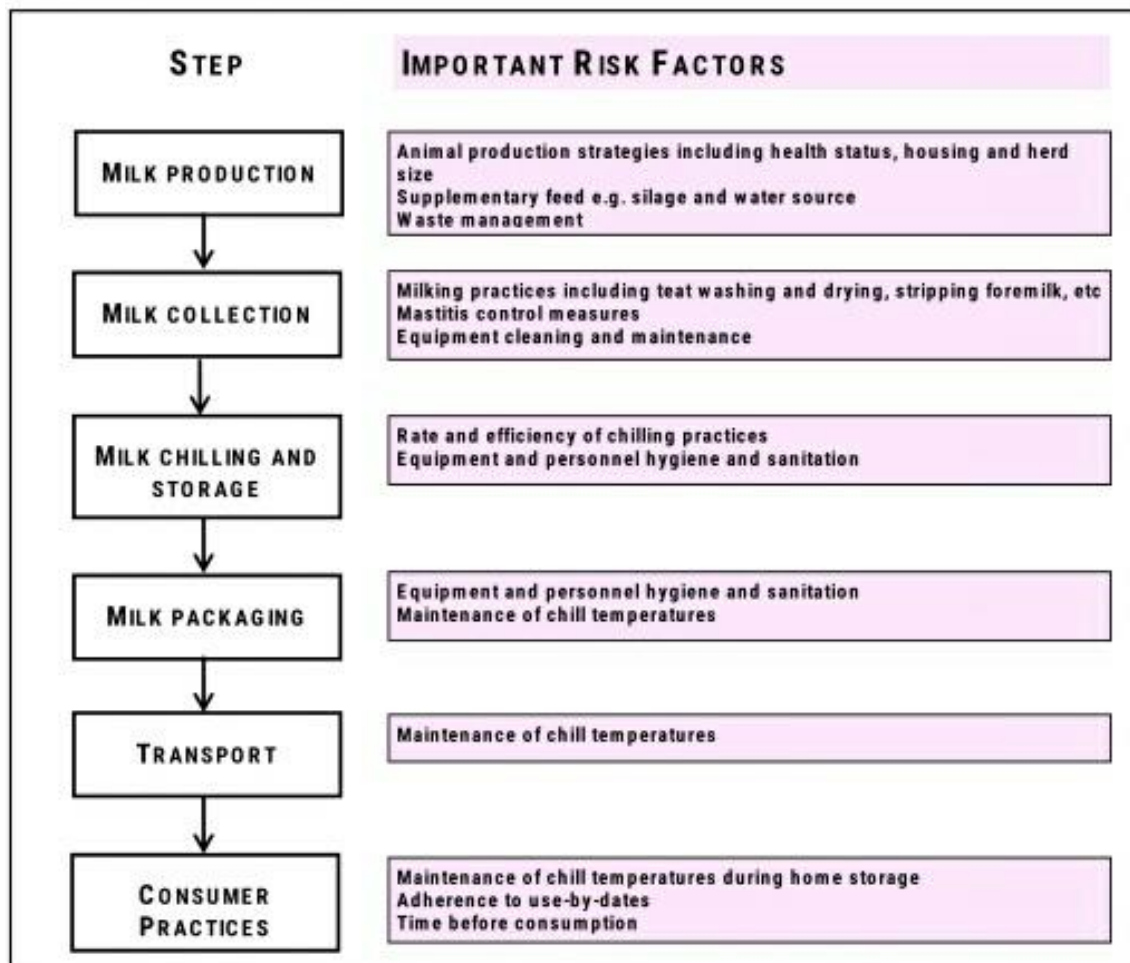
The wellbeing of crude cow milk can be impacted by administration and control measures along the whole dairy production network. Control of creature wellbeing, adherence to great milking practices, and control over milking parlor cleanliness are critical in diminishing the microbial load in crude milk.

The displaying embraced exhibits that in spite of the fact that the pathogen level might be low in crude milk, there remains a danger of causing sickness if expended. The capacity to identify pathogens in crude milk relies upon the exactness of testing, aptitude of work force and the point of confinement of identification for particular testing systems and pathogens.

Pathogen defilement of crude milk might be decreased by practicing upgraded sterile control all through the milk gathering stage. Practices, for example, nipple washing and plunging, foremilk stripping, and great milking cleanliness will diminish the quantity of living beings (pathogenic and deterioration) that may enter the milk from ecological sources.

Pre-milking udder washing with clean water and drying utilizing hand towels lessens milk defilement by transient microscopic organisms situated on the outside surfaces of the udder.

Post-milking nipple sterilization lessens the inhabitant nipple skin bacterial populace, which is the primary wellspring of disease for the mammary organ.



All the steps associated with the milking and collection procedure can be discussed targeted by the questions like

- (1) What are the dangers to general wellbeing and security postured by the utilization of crude bovine milk?
- (2) What are the components that would have the best effect on general wellbeing and security along the generation chain?

SCOPE OF THE STUDY

The reason for this evaluation is to give an accessible logical information on the general wellbeing and dangers related with the utilization of crude dairy animal's milk; and to give counsel on methodologies which might be utilized to affect on any distinguished hazard.

The extent of this hazard evaluation is to survey the hazard to general wellbeing and security from drinking crude dairy animal's milk. Surveying the dangers coming about because of utilization of further handled crude milk items is outside the extent of this appraisal.

Albeit additionally handled items are outside the extent of this hazard appraisal, the discoveries might be utilized to decide the dangers related with the crude milk proposed for handling into other crude milk items.

This study is targeted to the identification of possible source and types of microbial contaminates of milk and characterization of harmful bacteria developed during improper storage conditions, prolonged transport and various temperatures before processing of milk in milk plants.

OBJECTIVES OF THE STUDY

- **Identification of possible source of contamination of milk**
- **Identification of different types of contaminants of milk**
- **Characterization of different possible harmful bacteria found in milk**
- **Analyze and estimate the time of growth of different bacteria**
- **Effects of alteration of time, storage conditions and temperature on growth of bacteria identified in milk**

REVIEW OF LITERATURE

Nourishment of harmful bacteria's quality can be sustenance's by its load and rate (Rosmini et al., 2004), and creation of its clean states (Guerreiro et al., 2005). Looked into writing demonstrated poor microbiological nature of crude drain because of bacterial defilement (Ahmed and Abdellatif, 2013), deficient bundling framework (Singh et al., 2012) and despicable control of temperature (Moussa et al., 2013), which support microbial development and digestion and acquires unwanted changes in this manner abbreviate the timeframe of realistic usability of drain (Fromm and Boor, 2004). Assorted handling strategies like thermization, Low Temperature Long Time (LTLT) sanitization, High Temperature Short Time (HTST) purification, cleansing, ultra high temperature treatment (Gedam et al., 2007), bright treatment (Reinemann et al., 2006; Matak et al., 2007), microwave treatment (Tremonte et al., 2014), film preparing (Eckner and Zottola, 1991) and microfiltration (Elwell and Barbano, 2006) is used for the treatment of crude milk for good being utilization by decreasing harmful bacteria's leads to upgrades the time span of usability by inactivating deterioration microorganisms. Among different methods, sanitization is generally embraced however it isn't intended to disinfect drain and microbiological nature of purified drain is represented by the underlying vegetation of crude drain, the handling conditions and post-warm treatment tainting.

Satisfactory sustenance control program must be actualized in all nations around the globe to guarantee safe nourishment with worthy nutritious traits at a reasonable cost. Dairy items must conform to the microbiological criteria set around the administrative experts, which could be accomplished through purification or more serious warmth medications and counteractive action of post-warm treatment pollution. In the recent time, endeavors had been lead to feature purified drain by its microbial reflection.

Blendation of milk in specific mammary organ cells and antiseptic when emitted into the alveoli of the udder and might be debased amid draining and taking care of with hardware, staff and ecological sources and may contain pathogens. Generally, sensation embraced drain procedure to guarantee totally decimation of all harmful and deterioration microbes, normally found in drain and inactivation or lessening of other harmless waste microbes and unwanted chemicals to shield the sustenance estimation of drain.

The characterized sanitization as "A microbiocidal warm treatment went for decreasing the quantity of any pathogenic microorganisms in drain and fluid drain items, if exhibit, to a level

at which they don't constitute a huge wellbeing peril. Sanitization conditions are intended to viably decimate the living beings *Mycobacterium tuberculosis* and *Coxiellaburnetii*". At first, sanitization conditions were contrived to inactivate *M. tuberculosis* however in this way, *C. burnetii* showed up as the most warmth safe creature introduce in the drain and subsequently, purification was upgraded to accomplish no less than a 5-log diminishment of *C. burnetii* in entire drain. The HTST sanitization of pathogens and is successful in decreasing the reasonable populace of *Mycobacterium avium* subsp. *paratuberculosis* however its viability relies upon the aggregate feasible fixation (Okura et al., 2012). Stabel and Lambertz (2004) noted more prominent warm devastation because of sanitization of Ultrahigh-temperature drain vaccinated with higher focuses of *M. paratuberculosis*, paying little heed to time-temperature blends embraced. Amid purification the underlying fast decrease in populace of *M. paratuberculosis* in bovine's drain isn't because of warm demise however ascribed to collection of cells into clusters. Sanitization proficiency can be controlled by Phosphatase test. Basic phosphatase, a compound normally introduces in drain of all well evolved creatures have a warm protection more prominent than that of the most warmth safe non-spore-framing pathogens ordinarily found in drain (Sharma et al., 2003) and henceforth, its devastation affirms appropriate sanitization. Positive Phosphatase action is the demonstrative of deficient purification or tainting of sanitized drain with crude drain or post-process bacterial pollution.

Microbiological investigation of sanitized drain showed nearness of pathogens like *Staphylococcus* sp., *Salmonella* sp. (Singh et al., 2011), coliform from India, *Salmonella* from Nigeria, *Enterobacter* spp., *E. coli* from Japan, *Staphylococcus aureus* from Brazil, coliform, *B. cereus* from Kuwait deactivation of phosphatase and *Salmonella* sp. in any case, nearness of coliform in 57.5% examples of purified drain from Brazil.

Nearness of *Salmonella* in sanitized drain because of disgraceful purification coming about because of breaking down of a pasteurizer valve and post-sanitization tainting of sanitized drain with *Bacillus cereus* from bundling paper and board and filling machine have additionally been accounted for. Murphy (1997) ascribed unclean hardware, ill-advised sterilizing rehearses and millstone statement for higher aggregate reasonable tally in research center sanitized drain. In this manner, to guarantee safe purified drain appropriate purification and avoidance of post-sanitization defilement is essential.

Crude dairy animal's milk for human utilization is as of now not allowed available to be purchased consequently particular creation and industry data is inaccessible. It has in this

manner been expected that at the very least, practices, techniques and directions relating to the current cow-like dairy industry would likewise apply to the creation of crude cow milk.

The hazard appraisal considered residential and global data from distributed and unpublished sources on: milk generation frameworks, commonness and levels of pathogens in crude dairy animals milk and in cows, utilization information, and epidemiological information. This data gave a general photo of the general wellbeing dangers related with utilization of crude dairy animals milk.

Understanding the degree to which cows in the crowd convey pathogens in their gut, and shed them in their defecation, gives a vital method for taking out transporter creatures and decreasing the pathogen stack in the ranch and milking condition. Powerful administration of this would require abnormal state veterinary supervision and continuous observation of individual creatures in a crowd. Such measures include critical and regularly escalated mediations with attendant authorization specialist oversight.

The appraisal draws upon the discoveries of the Profile which distinguished microbiological dangers in crude milk, essential creation hones and other data significant to crude bovine milk. The evaluation talks about microbiological dangers related with crude dairy animals milk, their attribution to crude milk interceded foodborne ailment and the essential generation and handling factors which affect on crude milk wellbeing.

The evaluation depends on the Codex hazard appraisal system and uses the yields of quantitative displaying which gauge the hazard per irregular every day serve of crude milk to purchasers from enterohaemorrhagic E. coli, Listeria monocytogenes, Salmonella spp. also, Campylobacter spp. display in crude dairy animals milk. The model uses information including all phases from milking through to utilization.

Utilization of crude dairy animals milk on school camps, amid cultivate visits or by means of utilization of items promoted as pet or restorative milk, has been involved in eight episodes of sickness between 1998 2003

Other than the weight of sickness information, the quantitative displaying established that:

- Increased utilization of crude dairy animals milk relates to an expansion in the anticipated number of ailments

- Inclusion of deterioration in the model brought about a general reduction in the quantity of anticipated ailments
- Growth of pathogens happened overwhelmingly amid household transportation and capacity of crude milk
- The event and constancy of disease inside groups or individual creatures is frequently discontinuous, which demonstrates hard to identify and routinely screen
- The capacity to recognize low levels of pathogens in crude milk is restricted unless exhaustive examining plans are utilized

The hazard appraisal of crude dairy animals' milk unites data on the general wellbeing dangers related with the utilization of crude bovine milk, and gauges the subsequent weight of ailment that may happen under current generation and promoting conditions.

With a specific end goal to gauge the probability of ailment for purchasers following utilization of crude dairy animals' milk, quantitative microbiological displaying was embraced. The demonstrating anticipated the quantity of sicknesses per 100,000 servings of crude milk devoured specifically from the mass milk tank, after homestead door deal, and retail deal for four recognized key milk borne pathogens: *Campylobacter* spp., enterohaemorrhagic *Escherichia coli*, *Salmonella* spp. what's more, *Listeria monocytogenes*.

The key discoveries of the hazard evaluation can be compressed as:

- Raw dairy animals milk is related with foodborne ailment globally, and has been connected to sicknesses.
- Four key pathogens are related with flare-ups of foodborne disease embroiling crude cow milk, these are *Campylobacter* spp., *Salmonella* spp., *Listeria monocytogenes* and pathogenic *Escherichia coli*.
- Internationally, crude bovine milk has been observed to be a huge wellspring of pathogenic microorganisms.
- Unpublished research proposes utilization of crude milk is probably going to be low among the all-inclusive community, notwithstanding, certain gatherings specially devour crude milk.
- The weight of ailment after retail buy was anticipated to be under 1 instance of campylobacteriosis, 97 instances of EHEC, 153 instances of salmonellosis and up to 170

instances of listeriosis (in a vulnerable sub-populace). The evaluated number of cases are per 100,000 every day serves of a mean day by day admission of 540 ml of milk to a tyke.

EQUIPMENT, MATERIALS, AND EXPERIMENTAL SETUP

Equipment's

Plasticware

- Cotton
- Microtips 1000ul
- Microtips 200ul
- Microtips10ul
- Microcentrifuge Tubes (1.5ml)
- Microcentrifuge Tubes (2ml)

Glassware

- Conical Flasks (250ml)
- Conical Flasks (1000ml)
- Conical Flasks (100ml)
- Culture Test Tubes
- Petri Plates
- Glass Beaker (200ml)
- Glass Beaker (500ml)

Materials

Media

- Simmons Citrate Agar
- Christensen's Medium
- Triple Sugar Iron Agar
- MR-VP Media

- Sorbitol MacConkey Agar
- XLD Agar
- Campy CVA Agar
- BD Yersinia Selective Agar
-
- Bromothymol Blue
- Sodium Carbonate
- Disodium P-Nitrophenyl Phosphate
- Kovac's Indole Reagent

Experimental Setup

- Autoclave
- Laminar Air Flow
- Incubator
- Microwave Oven
- Weighing Machine
- Water Bath
- Refrigerator

RESEARCH METHODOLOGY

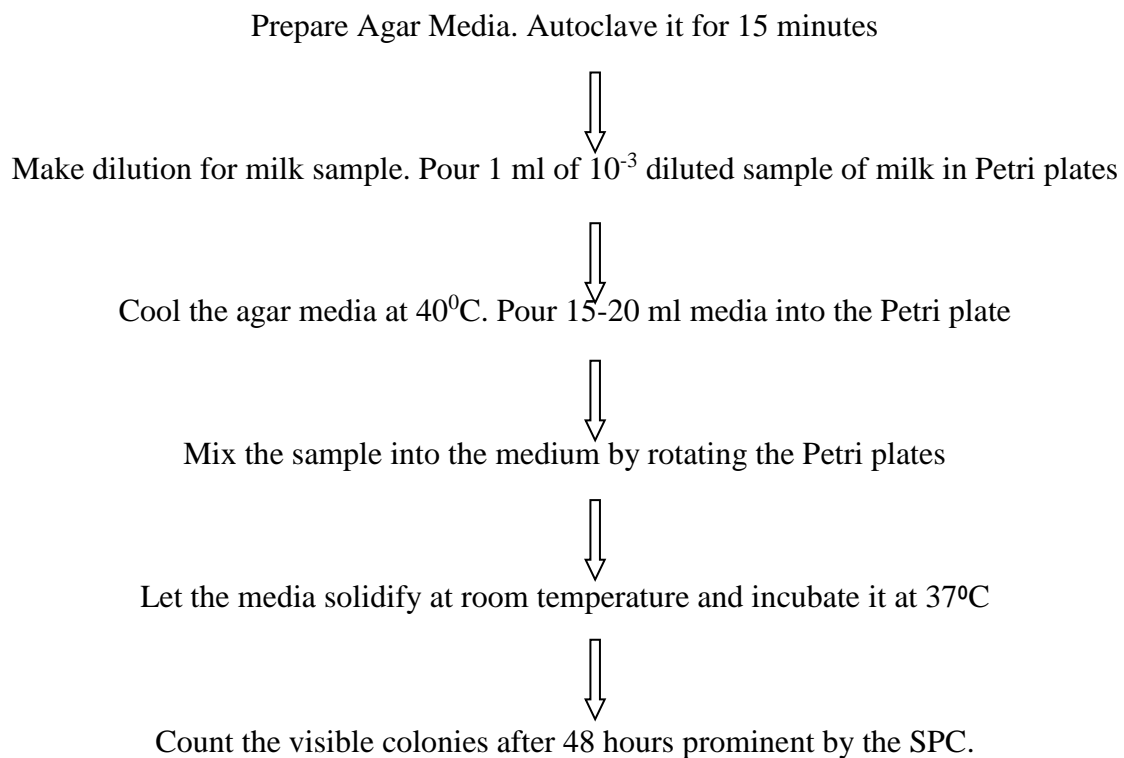
Bacterial Tests in Raw Milk

The Standard Plate Count is utilized broadly into administrative and premium testing plans. Notwithstanding this test, crude milk will be conducted to various bacterial tests which utilized that how that milk was delivered. Such tests might be incorporated into deciding qualification or may be utilized to ensure additional effective affirmation instrument. The bacterial tests frequently utilized as a part of expansion to the standard plate count are the Preparatory Incubation Count, the Lab Pasteurization Count and the Coliform Count.

Standard Plate Count Test

This method is generally used for all types of milk products but basically used for the examination of raw as well as pasteurized milk. Generally, milk supply quality can be determined by this method. I have done this method with different kinds of media's such as agar, nutrient agar and MacConkey agar. All the media's have their own identification of specific bacteria present in the milk such as

- Agar is used for enumeration of bacteria in milk and milk products.
- MacConkey agar used as an isolation of gram negative bacteria present in milk such as *E. coli*
- Nutrient agar is used for the isolation of lactic acid bacteria such as *Streptococcus thermophilus* and *Lactobacillus bulgaricus*.



Preliminary Incubation Count

The Preliminary Incubation Count (PI) gives milk creation hones. Strategy includes the milk asset at 55°F for 20-hour preceding media plate. Progression energizes, gathering of develop microorganisms which will develop at low temperature. Microscopic organisms in the brooded

test checked with the standard plate count technique than contrasted with previous test from the same test to decide whether a huge increment has happened. PI tallies are by and large higher than the count. Tallies with a 3-4-crease increment are viewed as critical. Some consider tallies more noteworthy than 50,000 cfu/ml to be of matter paying little respect to the SPC, however now and again the tallies might be equivalent in uncommon cases this count is lower.

As large preliminary incubation count is regularly connected to completely perfect sterilize as the milking framework or the bovines. Microscopic organisms thought to be characteristic verdure of the dairy animal, accept those that which have mastitis and which are not able to develop at the preliminary incubation temperature. Be that as it may, PI equivalent marginally larger than a high SPC might recommend it is perhaps because of pathogen present in mastitis. Minimum cooling time or delayed capacity might likewise bring about inadmissible PI levels which permitting creatures that develop at hatchery temperatures to duplicate.

Lab Pasteurized Count

Despite the fact that most microorganisms are demolished by purification, there are sure sorts, specific stages of bacteria which are most certainly not. The Lab Pasteurized Count shows the survival of bacterial quantity in sanitization procedure. Milk tests are warmed to reproduce clump purification at 145°F for 30 minutes. Microscopic organisms which are exist in sanitization are at that point tallied utilizing the SPC technique. LPC are for the most part lesser than SPC, with tallies more noteworthy than 300 considered high. In common vegetation of the dairy animals, microorganisms additionally those related with mastitis, are for the most part not thermoduric. High LPC esteems are regularly related with an unending or relentless cleaning disappointment in some range of the framework or huge levels of tainting from dirtied dairy animals. Other regular reasons for large LPC are pumps that are cracked, pipeline gaskets, swellings, elastic parts & milk stone stores.

Coliform Count

The Coliform Count (Coli Count) system gives those microscopic organisms that are generally normally related to natural defilement. Milk tests can be done on media plates that energizes coliform microbes for their development, while keeping the development of remaining. In spite of the fact that coliforms are regularly utilized as markers of fecal sully, certain strains already exist in the earth. Microbes of coliform enters in the milk supply as a result of the hook getting to be plainly dirty with fertilizer amid milking. By and large, checks more noteworthy than 100 cfu/ml should demonstrate low quality milking cleanliness different wellsprings of

tainting. greater coliform checks all the more frequently result from filthy gear and in uncommon cases come about because of milking bovines with natural coliform mastitis.

Methylene Blue Reduction Test

The method is based upon the ability of microorganism to lower the oxidation reduction potential of the media. The change in oxidation reduction potential is detected by the change in the color of dye.

- The dye in media is blue in color in oxidized state
- Become colorless in reduced state

Greater number of microorganisms in milk, shorter time for reduction of methylene blue.

MBRT- The time taken by dye to become colorless is known as Methylene Blue Reduction Time.

I have taken two samples, one fresh milk and one sample before 3 days. A sample before three days is not sinked. There will be only change in their texture. Take 2 test tubes, add milk sample in each test tube (10ml). Put 1 ml of methylene blue to each test tube then invert the tubes. And further, put them into water bath at 37⁰C for 8 hours and results will be observed. The fresh milk sample has changed their color and turns white i.e. the fresh milk has good quality as compared to 2nd sample.

Precaution

Invert the tubes after every hour of incubation otherwise microorganisms will float to the top with the cream in the milk sample.

The Resazurin Test

The resazurin test is led like the methylene blue diminishment test as judgment of value construct in light of shading created after an expressed time of brooding as well as on the time required to reduce the color to a given specific point. Various alterations has been proposed. The two most basically applied are the "one-hour test" or the "triple-perusing test" taken few hours of hatching. Different adjustments has an incentive in particular applications.

The procedure for the resazurin test is as per the following: Prepare resazurin arrangement by dissolving one resazurin tablet in specific volume that is 100ml to 200ml of hot refined water as is done in the methylene blue test. Place specific volume of color arrangement in a sterile test tube, at that point include 5ml-10ml of test. Close the tube with plug, take to the hatchery

and, when the temperature achieves specific temperature, blend the modify milk and color. Hatch at 36o C. Tubes are characterized toward the finish of an hour in the "one-hour test" or toward the finish of three progressive hourly interims in the "triple reading test." The accompanying connections from shading or character are by and large acknowledged:

Sample shade: Milk Quality

Blue (no change in shading): Excellent

Blue to profound mauve: Good

Profound mauve to profound pink: Fair

Profound pink to whitish pink: Poor

White: Bad

The resazurin test may be a significant efficient apparatus if appropriately led and shrewdly deciphered, however ought to be build up by tiny checking.

This test is a problematic file of bacterial quality in milk accomplished by different reports. A noteworthy feedback of the technique is that the resazurin diminishment time of refrigerated packaged milk at either 20o or 37o C is much too long to be of any an incentive in assessing bacteriological decay of put away milk.

Standard methods take notes by no means shall consequences of Reassuring tests and methylene blue test be accounted for as far as bacterial numbers.

EXPECTED OUTCOMES

Showcasing in crude dairy animals milk for human utilization, in spite of the fact that progressing illicit offer of the item happens in numerous purviews.

The framework for delivering crude milk would be relied upon to be like that utilized for creating dairy animals milk for purification. Be that as it may, the size of operation would more probable be small scale/boutique. Bundling of crude milk would be relied upon to happen on cultivate or at offices near the generation condition with a limit with regards to isolating milk planned for crude or purified item.

The microbiological status of milk measures utilization to characterize milk for sanitization should likewise applied to milk so that sold in the crude shape. The incorporate industry best practice administrations, for example, control programs for mastitis, initial and final milking udder treatment rehearses, milking parlor cleanliness plans.

An extensive variety of pathogens has been found in crude milk, and has been in charge of effecting flare-ups of diseases related to food.

In 1976, a huge flare-up of Salmonella Typhimurium PT9 happened including more than 450 cases. The larger part of patients was under age of 12-15 years and most were under 9-10 years. Attributes related to flare-up recommended particular source and most of the patients gave a past filled with devouring crude milk. Ensuing examinations detached S. Typhimurium PT9 from 7 of the 275-people researched and 10 tests of mass and container unpasteurized milk gathered ahead of schedule in the episode.

It is recognized that flare-ups containing 101 instances of sickness related with the utilization of crude cow milk. Campylobacter spp. is the most widely recognized specialist study for the cause of diseases, with Cryptosporidium spp. also, S. Typhimurium PT44 representing one flare-up each. There is one flare-up accompanying obscure etiology.

Many flare-ups happened on various camps where unpasteurized milk was expended, while two episodes ensnared unpasteurized milk devoured on ranches. Unpasteurized milk was likewise devoured and prompted flare-ups in a group setting and in a school. The programs were explored utilizing 3point source studies and one case control examine. Previous years information up to 2001 does not distinguish flare-ups and does not know their identification.

Universally, crude cow milk utilization has regularly been related with sickness related to food. Between 1992 - 2000, 2% were milk-borne diseases, with the larger part approximately more than 50% credited to the utilization of crude milk.

More of the late, episodes of *S. Typhimurium* happened including 60 and 28 cases separately. The *E. coli* O157:H7 is in charge of an episode including 5 cases of disease including 2 more cases causing haemolyticureamic disorder. The middle period of cases in this episode was 8 years that might had expended crude milk from the common dairy. *E. coli* O157:H7 is likewise in charge of an episode in 2005 in which 20 individuals turned out to be sick. Maximum patients associated with a group plans and have expended crude milk.

Specialist study of disease normally connected with crude milk interceded ailment incorporate *S. Typhimurium*, *E. coli* O157:H7 and *Campylobacter* Spp. *Listeria* spp. furthermore, other *Salmonella* serovars, accounted for.

A current efficient researched the quality of confirmation to help causal connections between sickness with crude milk utilized items. Basic discovery describes direct proof exists to help a causal connection between utilization of crude milk items and *Salmonella* species, *E. coli* spp., *Listeria monocytogenes* and *Campylobacter* spp.

Examined wellsprings of diseases large decided through epidemiological and additionally microbiological affiliations. The capacity to distinguish an episode through the current reconnaissance framework is basic to empower an examination to continue. Troubles exist in distinguishing and ascribing ailment to a specific nourishment include:

- Gathering of sustenance utilization histories
- Take much time in acknowledgment or warning of a flare-up
- Not able to follow sustenance items from the source
- Not much interest of people with examinations, especially acquired nourishments which are not allowed to be in market lawfully
- Particular pathogen takes much time to present. (e.g. *Listeria monocytogenes*)
- Not able to acquire agent nourishment tests for investigation
- An absence of accuracy in or appropriate strategies for test investigation and pathogen recognizable proof

Recognize that flare-up information just speaks to a little extent of real instances of foodborne disease, the same number of episodes go unrecognized and additionally unreported to wellbeing specialists. Individuals don't generally look for restorative consideration for mellow types of gastroenteritis, medicinal experts don't generally gather examples for investigation and not all foodborne ailments expect notice to wellbeing specialists.

PROPOSED WORK PLAN WITH TIMELINES

Workplan	Timeline
Visit to various industries	February to march 2017
Discussion with specific industry	March
Review of literature	April to may
Training in specific industry	June to July
Review of methodology	August to September
Standardization of methods	October to November
Collection of sample from various sources and performance	January to April

RESULT AND DISCUSSION

Microscopic organisms develop in extremely different conditions, which clarifies why they are discovered almost wherever on Earth. Despite the fact that microscopic organisms are great at adjusting to their surroundings, certain conditions advance bacterial development more than others. These conditions incorporate temperature, dampness, pH and ecological oxygen. Understanding the ideal conditions for bacterial development can conceivably enable you to decrease your hazard for bacterial contaminations and sustenance harming.

Warm Temperature

Most ailment causing microbes flourish in warm temperatures, particularly those near body temperature. The human body, along these lines, gives a perfect domain to many sorts of microorganisms to develop. Certain strains of microscopic organisms, notwithstanding, can develop at lower or higher temperatures. Since perfect temperature is critical for the development of any given types of microorganisms, nourishment must be dealt with properly to maintain a strategic distance from sustenance harming. As a rule - however not all - refrigerating or solidifying nourishment is adequate to smother the development illness causing microscopic organisms, for example, Staphylococcus. Completely cooking meats and poultry to the right inside temperature is additionally vital to execute unsafe microscopic organisms that might be available in the nourishment, for example, Salmonella and E. coli.

Moisture

Microscopic organisms require water to develop and kick the bucket without a water source. Wet zones are especially inclined to bacterial development, for example, washrooms and

kitchens. Water content in sustenance additionally gives an amazing domain to many sorts of microbes to develop. Certain sustenance's can be dried out or solidify dried, which expels the vast majority of the water and can take into account longer stockpiling without bacterial development. Soggy tissues in the body, for example, the mouth and nose, give an incredible wellspring of dampness for microorganisms and are especially inclined to bacterial development.

Environmental pH

The pH of a situation - a measure of its causticity or alkalinity - is critical for bacterial development. Most strains of infection causing microscopic organisms like to develop in conditions with a close unbiased pH, like the pH of the human body. A few strains of microorganisms, in any case, can live in more acidic or more basic conditions. Cleaning arrangements are ordinarily profoundly acidic or fundamental, which eliminates microscopic organisms, since they can't make due at these extremes of ph.

The causticity of sustenance is additionally a vital factor influencing bacterial development. More acidic sustenance's can ordinarily be put away longer without ruining. Safeguarding specialists that expansion the acidity of nourishment, for example, citrus extract, are normally added to help anticipate bacterial development and take into consideration longer stockpiling. Vinegar and lemon juice have a comparative impact.

Environmental Oxygen

The nearness of oxygen can significantly influence the development of microscopic organisms. Many sorts of ailment causing microbes develop best in an oxygen-rich condition or expect oxygen to develop. This is the reason numerous business sustenance's are vacuum-fixed. Vacuum fixing - otherwise called decreased oxygen bundling - restrains the development of many sorts of microscopic organisms and growths that reason nourishment waste. Once the vacuum seal is broken, presentation to nature and oxygen confines the time span of usability. Keeping sustenance legitimately fixed while amid capacity is a decent preventive measure against bacterial development since it limits the measure of oxygen. Legitimate fixing is additionally essential while doing home canning for comparative reasons.

While decreased oxygen represses the development of many sorts of microbes that can ruin nourishment, there are others that flourish without oxygen. Two essential illustrations are Clostridium botulinum - the microscopic organisms in charge of botulism - and Listeria

monocytogenes, another nourishment borne microbes that is especially destructive to pregnant ladies, infants and individuals with a debilitated insusceptible framework.

EXPECTED RESULT

Counting of the Colonies

After hatching of the considerable number of plates, tallies of the quantity of settlements in every plate was finished with count counter. A mean of the tally is acquired than duplicated along the fitting weakening component. The mean consider was ascertained demonstrated as follows.

Mean = Total Viable Count

The estimation of suitable number of organisms in every example is made in colony forming units.

TVC = 1

Sample weight *N*D

Where N = average number of colonies

D = dilution factor

Isolation of Microorganisms from Sample

Specimens were weakened in dilution of 10 times serial expressed previously. The brooding part of plating is utilized by pour plate technique. From the 10-3 weakening tube Put 1ml into a few sterile Petri plates by the sterile pipette. Liquid Nutrient agars, MacConkey agar, and Sharpe agar, mannitol salt agar and potato dextrose agar used to fill the plates. All plates were permitted to settle in the plates. Each plate is copied.

The agar media plates were moved into a refrigerator at 25oC for 3 – 5 days while the other supplement plates test is copied.

Again, the media agar plates are allowed to be in a refrigerator at 25oC for 3 – 5 days while the other supplement plates allowed to be into incubator at 37oC for 18 – 24 hours. The media plates were inspected day by day settlement development.

Sub- Culture and purification

Afterwards the incubating period, discrete settlement in microbe's plates are picked with a blazed wire loop and refined on recently arranged supplement media plates. Additionally, flared cut is utilized to culture different shade of mycelia development from agar plates onto a recently new media plates. Plates fittingly properly. Every single supplement plate is moved into a hatchery at 37oC for 16 - 48 hours while all the PDA plates were brooded at 25oC for 4 – 6 days. Cleaned settlements moved to inclines & put away appropriately from facilitate portrayal.

Characterization of purified Culture (Bacteria)

Every sanitized bacterium is inspected visibly as settlement structure, infinitesimally afterward decide to recolored the gram status. Portrayal of segregation of bacteria is done by various standard biochemical tests. Maturation test of sugar were likewise complete and then recorded. The outcomes coordinated with a bacteriology. The biochemical tests including Catalase, Indole, methyl red etc.

INTRODUCTION

Milk is the principal nourishment of all newborn children. So it assumes a fundamental part in our eating routine for the duration of the life. Milk has a high nutritive esteem and contains all the fundamental segments of our eating regimen like proteins, fats, vitamins and minerals. It is the main single finish sustenance for a well being and the development of individuals in nature. In India, dairy animals and wild ox are most essential for business creation of milk and milk items. This is the reason, why cow is considered as a hallowed creature and even loved and worshipped in India.

Individuals of all age bunches consume milk. In created nations, the utilization of milk and milk items is higher when contrasted with the restricted utilization in creating nations. This request is becoming higher in created nations because of the expanding population, ascend in pay, urbanization and the adjustment in dietary propensities. As indicated by the Food and Agriculture Organization of the United Nations, the utilization of milk and milk items is by more than 6 billion individuals around the world. The lion's share of these individuals have a place with creating nations. In India, around half of the milk is expended on-ranches.

Milk and milk items are accessible on shops and dairies. There is countless number of items that are delivered from milk and these change up our dinners. Cheddar, curd, spread, cream, khoa, rabri, frozen yogurt and so on are produced using milk. It is utilized at both family and mechanical levels. Ensure that the essence of milk is held amid the creation and capacity.

Milk is accessible in both new and handled structures. The previous being sold as fluid milk, drawn from the udder of the creature or after the gathering and sanitization to make it alright for human utilization.

A few people deliberately add particular substances to the milk to decrease its virtue which is named as milk defilement. At the point when the shoppers need to get the greatest amount at a cost as low as could reasonably be expected, the merchants must address the issues of the purchasers. Merchants at that point offer the milk of second rate quality and subsequently they debase the milk. An adulterant is any substance which is utilized to decrease the nature of the item and to receive most extreme advantage in return.

Debasement might be deliberate or inadvertent. Accidental defilement is for the most part because of obliviousness, carelessness or absence of legitimate offices. Accidental sully may likewise happen because of small scale living beings. Some bundling perils are likewise there. The source may likewise be an infected creature. On the off chance that the creature is infected, milk is additionally not beneficial. It additionally relies upon their encourage. Presently, milk is first prepared in enterprises. There it originates from various sources and afterward blended. Individuals intentionally add adulterants to get most extreme advantage. They don't consider the unsafe impacts that these substances can cause. Such individuals have lost their good and otherworldly esteems. There must be strict laws to rebuff these individuals.

SUMMARY AND CONCLUSION

While the acquaintance of sanitization has assisted with guarantee the security of dairy items, advance have been slower in keeping the waste of microbe's cheddar also, milk items. Overall institutionalized sanitization practices would be a viable initial phase in dispensing with or decreasing the levels of numerous decay microorganisms. Notwithstanding, averting post process tainting by deterioration microorganisms furthermore, hindering the development of surviving life forms be a test. Advancements or additives are expected to keep the development microorganisms which are decayed furthermore, expand the timeframe of realistic usability of dairy items. Restricted relevance of current endorsed antimicrobials, for example, sorbic corrosive and natamycin gives a noteworthy chance to grow the weapons store of additives accessible for the present dairy processor. Moreover, concentrates to decide the connection of current additive innovations against waste microorganisms are additionally required. Enhanced strategies for recognizing deterioration organisms, particularly the moderate developing psychographs and parasites, could help with finding the specialty situations in handling offices that prompt post process pollution. The current time will convey many difficulties to the milk industry, however keeping up the effectiveness and timeframe in realistic usability of this very nutritious nourishment ought not be one of them.

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